Practical training for substrate preparation techniques for mushrooms

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Introduction

The culture media (substrate) for mushrooms is as important as soil is for plants. Mushrooms are the fruiting bodies of macro-fungi. Fungi lack chlorophyll, which makes them different from plants that obtain energy from the sun through photosynthesis. Instead, mushrooms can grow on ligninocellulosic wastes, degrading and taking nutrients from those materials for their growth. Agro-wastes or leftovers either from agricultural practices from forest stands, such as cereal straws are largely burnt or discarded by the farmers. However, these from agro-wastes can be further treated and recycled as useful raw materials for use in the cultivation of value-added edible mushrooms. This type of bioconversion by mushroom mycelia exercise can greatly reduce environmental pollution (S. T. Chang 2007). Therefore, mushroom cultivation is a friendly green technology for extending recycling use of agro-wastes/leftovers and eco-friendly resource management beneficial to the environment.

Preparation of substrate materials and additives

Commonly used substrate materials for mushroom cultivation are paddy straw, sawdust, cottonseed hull, cereal straw, corncob, coffee waste, sugar cane bagasse, wild grasses, and other plant fibers with high lignin or cellulose contents (K. Hyungjong and Byung S. K. 2004). These culture substrates are considered agricultural wastes, as substrates are very inexpensive and sometimes free.

Various edible mushrooms can be grown on different agricultural wastes and leftovers which, if not used, usually end up discarded, such as growing oyster mushrooms on a wider variety of substrates than any other cultivated mushrooms. Readily available additional nitrogen sources such as wheat bran, rice bran, or corn are necessary for mushroom growth during their vegetative growth stage. Additional substrates typically affect mushroom yield. In addition, gypsum, calcium carbonate, or chlorinated lime is added to the substrate and function as buffers for controlling pH value in the substrate.

Sawdust is currently the most commonly used basic substrate for mushroom cultivation, except for some mushrooms cultivated on fermented substrates (ie. the white button mushroom). Various types of trees available in each region can be used as sawdust sources. The frequently used hardwood tree species for mushroom cultivation include *Trema orientalis, Machilus kusanoi,* oak, poplar, alder, maple, mango, elm, and etc. Selecting tree species that are both favorable for mushroom growth and that are easily available is important for mushroom production. Pine, cedar, and redwood trees should be avoided to use for as mushroom culture substrates because resins in sawdust inhibit mycelial growth and colonization.

Bag cultivation

Bag cultivation is the most commonly used method for mushroom cultivation in many countries around the world. Plastic bags (polythene and polypropylene) are most frequently used for mushroom cultivation.

Sawdust preparation

Fresh sawdust is not suitable for mushroom culture because it can not absorb water immediately. To make sure the sawdust is moisten enough, the sawdust is piled up and the stacking needs to be turned over with water once every two months. After one to four months, the sawdust could be used for mushroom cultivation.

Additives :

Rice bran is a by-product of the rice milling process (converting brown rice to white rice).

Wheat bran is the hard outer layer of grain and consists of combined aleurone and pericarp.

Sample Formulation for *Pleurotus eryngii*

60% Sawdust (w/w), 24% rice bran, 15% wheat bran, and 1% calcium carbonate.

Substrate mixing

Sawdust is mixed with water and other supplements either manually or mechanically. During mixing, attention should be paid to evenly distributing water throughout the entire substrate. Generally, the moisture content of the substrate is typically adjusted to 65%. The palm test method is a simple way to check whether the mixture has proper water content.

Filling and compressing

The prepared substrate mixture is filled into bags before heat-treatment. The filled bags are then properly compressed for quick mushroom mycelia colonization. After filling and compressing, a 2–3 cm-diameter hole is made in the center, which allows for spawn down at the bottom of the bag. The hole provides a deeper inoculation and a better oxygen supply, enhancing faster colonization. Mushroom mycelia need some oxygen for respiration during growing stage; therefore, ventilative plugs or stoppers are recommended for free air

exchange. Farmers in Taiwan use plastic neck-rings and plugs with cotton balls to close the open–side of the bag.

Fig 1. The process of substrate preparation for bag culture: A) substrate mixing, B) substrates filling, C) holing, D) plugging with cotton balls.

Heat treatment

Before spawning, the substrate needs to be treated to kill or reduce pests and microorganisms by using sterilization or pasteurization. Sterilization kills all microorganisms while pasteurization only reduces the number of microorganisms. The sterilization method is recommended to minimize the risk of contamination.

Normal-pressure sterilization

Once the substrate bags are done, they are then placed into the autoclave or sterilizer. The desired temperature for normal-pressure sterilization is maintained at 100℃ for 6 hours, measured from the time the inside temperature reaches the target temperature. After sterilization, the substrate bags are then taken out and moved to a cooler room overnight.

High-pressure sterilization

The desired sterilization pressure and temperature are under the pressure of 1.5 kg/cm^2 and the temperature inside the sterilizer rises up to 121℃. When it reaches 121℃, the temperature is maintained for 60 minutes. After steam is released, the substrate bags are moved from the sterilizer to a cooler place. The sterilized substrates are cooled to room temperature for spawn inoculation.

Spawning

Mushroom spawn is a medium that carries mushroom mycelia. Most growers use spawn produced by themselves or commercial spawn providers. The following describes how to inoculate substrate bags.

Bottle cultivation

The bottle culture system has been successfully adopted for use in commercial production of spawn and mushrooms for many years in the past in Japan, Taiwan, Korea, and many other countries. The initial set-up cost for the system is higher for most beginning growers due to some automatic equipment and facilities are used. Some spawn suppliers favor propylene bottles since they are heat-resistant and thus, autoclavable, endurable, re-usable, and easy to handle (K. Hyunjon 2004). The major process of bottle culture system for mushroom production is illustrated as fig 1.

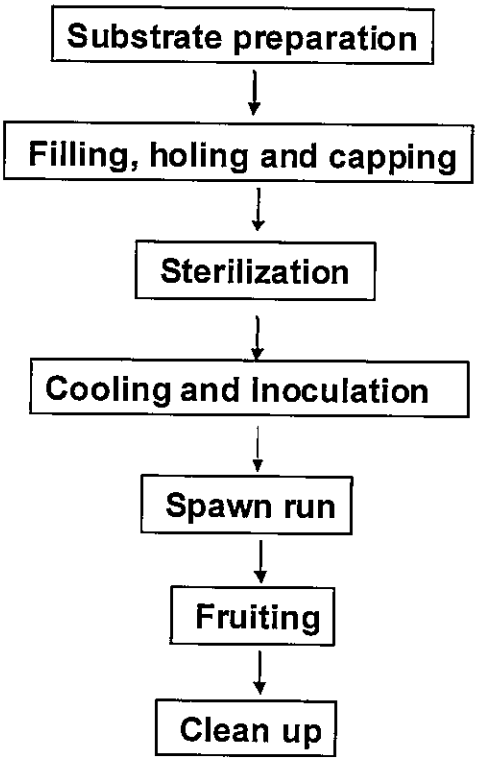

Fig 2. The major process of bottle culture system.

The section above describes **substrate preparation.**

Filling, holing and capping

The prepared substrate is loaded into the bottle feeder which fills bottles with the mixture. Once bottles are filled, compactors press the bottle mixture down to the pre-set height, and then hole-makers go through the compacted mixture. When the filling and the holing steps are complete, the bottles are transferred to another machine that automatically caps the bottles.

The section above describes **heat treatment (sterilization).**

Fig 3. The process of substrate preparation for bottle culture: A) sawdust stacking, B) substrate mixing, C) substrates automatic filling, D) holing, E) capping, F) bottles loading in the autoclave.

Cooling and Inoculation

After sterilization, the bottles are removed from the autoclave and cooled to 25℃ in the cooling room. Bottles ready to inoculate are moved from the cooling room through a small window onto the clean bench on the skate wheel conveyor. Before inoculation, the inside of the inoculating room must be disinfected with an ultraviolet lamp and 70% alcohol. The semi-automatic inoculating machine allows the operation to be much faster and effective.

Spawn run

Inoculated bottles are transferred to an incubation room, where the temperature and humidity are maintained at 20–21℃ and 65–70%. The spawn run is strain-dependant, and typically takes 30–35 days. During incubation, the maximum limit of CO_2 concentration for mycelia growth is 5000ppm.

Fruiting

Once the spawn run is complete, the bottles are brought to a growing room or exposed to a lower temperature. Low temperature or high humidity is controlled and maintained as the mycelia shift into reproductive growth from vegetative growth to induce fruiting body formation.

Clean up

After harvest, the bottles are loaded into an automatic machine to remove spent substrates. The emptied bottles are washed with water, dried, and arranged to be recycled for further use. The emptying area should be far from the growing and incubation room since the spent substrates might harbor pathogens or pest spores.

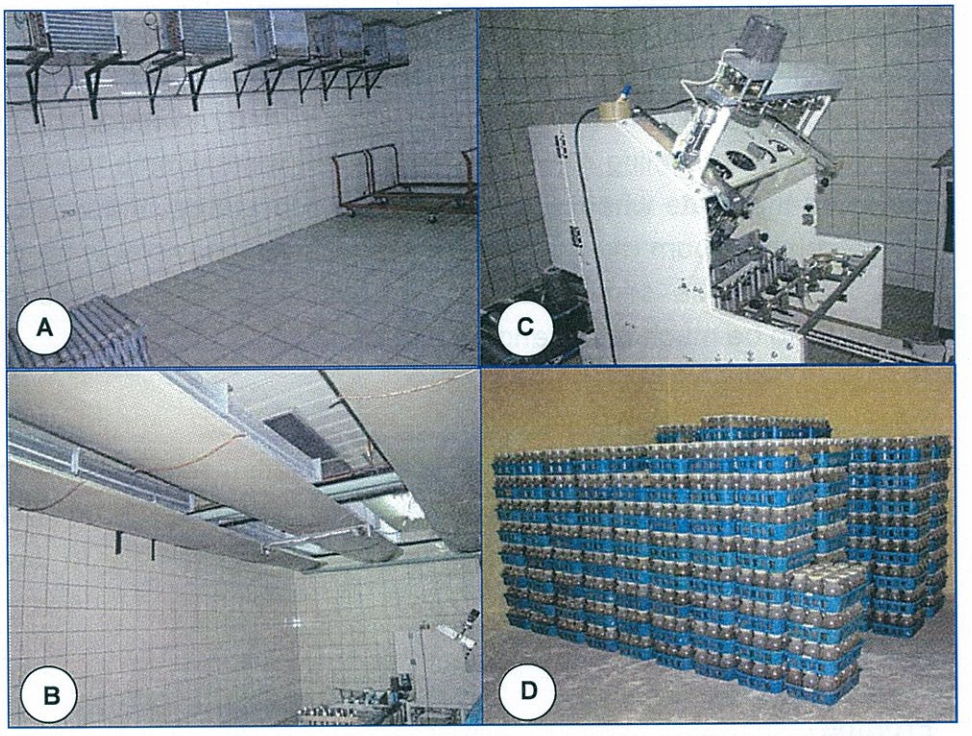

Fig. 4. A) A cooling room with mechanical cooling system, B) an inoculating room with air filter, C) a semi-automatic inoculating machine, D) incubation room.

Appendix

Common name: Enokitake, the winter mushroom, the golden mushroom

Scientific name: ***Flammulina velutipes*** (Curtis) Singer

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:4-16 ℃

Relative humidity: 95-100%

CO_2: 2000-4000 ppm

Light requirement: 20-50 lux

Fruiting body development:

Temperature:10-16 ℃

Relative humidity: 90-95%

CO_2: 2000-4000 ppm

Light requirement: 20-50 lux

Common name: Nameko, Slime Pholiota, Viscid mushroom,
Scientific name: *Pholiota nameko* (T. Ito) S. Ito et Imai
Growth parameters:
Incubation temperature: 24-29 ℃
Primordia formation:
Temperature:10-16 ℃
Relative humidity: 95-100%
CO_2: 500-1000 ppm
Light requirement: 500-1000 lux
Fruitbody development:
Temperature:12-18 ℃
Relative humidity: 90-95%
CO_2: 800-1200 ppm
Light requirement: 500-1000 lux

Common name: The oyster mushroom, Hiratake

Scientific name: *Pleurotus ostreatus* (Jacquin:Fr.) Kummer

Growth parameters:

Incubation temperature: 22-24 ℃

Primordia formation:

Temperature:10-16 ℃

Relative humidity: 95-100%

CO_2: ＜1000 ppm

Light requirement: 1000-1500 lux

Fruitbody development:

Temperature:10-21 ℃

Relative humidity: 85-90%

CO_2: ＜1000ppm

Light requirement: 1000-1500 lux

Common name: The Indian mushroom, the Phoenix mushroom,
Scientific name: *Pleurotus sajor-caju* (Fr.) Singer
Growth parameters:
Incubation temperature: 24-29 ℃
Primordia formation:
Temperature:21-24 ℃
Relative humidity: 95-100%
CO_2: 400-800 ppm
Light requirement: 1000-1500 lux
Fruitbody development:
Temperature:21-24 ℃
Relative humidity: 90-95%
CO_2: 400-800 ppm
Light requirement: 1000-1500 lux

Common name: king oyster mushroom,
Scientific name: *Pleurotus eryngii* (DC.:Fr.) Quél
Growth parameters:
Incubation temperature: 21-24 ℃
Primordia formation:
Temperature:16-18 ℃
Relative humidity: 95-100%
CO_2: 1000-2000 ppm
Light requirement: 20-50 lux
Fruitbody development:
Temperature:14-16 ℃
Relative humidity: 90-95%
CO_2: 2000-3000 ppm
Light requirement: 20-50 lux

Common name: Yuhuanmo, the golden oyster mushroom
Scientific name: *Pleurotus citrinopileatus* Singer
Growth parameters:
Incubation temperature: 24-29 ℃
Primordia formation:
Temperature:21-27 ℃
Relative humidity: 95-100%
CO_2: ＜1000 ppm
Light requirement: 500-1000 lux
Fruitbody development:
Temperature:21-29 ℃
Relative humidity: 90-95%
CO_2: ＜1000 ppm
Light requirement: 500-1000 lux

Common name: Brown swordbelt, the black poplar mushroom, Pioppino,

Scientific name: *Agrocybe chaxingu* Huang

Growth parameters:

Incubation temperature: 21-27 ℃

Primordia formation:

Temperature:10-16 ℃

Relative humidity: 95-100%

CO_2: ＜2000 ppm

Light requirement: 500-1000 lux

Fruitbody development:

Temperature:13-18 ℃

Relative humidity: 90-95%

CO_2: ＜2000 ppm

Light requirement: 500-1000 lux

Common name: The beech mushroom, Bunashimeji
Scientific name: *Hypsizygus marmoreus* (Peck) Bigelow
Growth parameters:
Incubation temperature: 21-24 ℃
Primordia formation:
Temperature:10-15 ℃
Relative humidity: 95-100%
CO_2: 500-1000 ppm
Light requirement: 400-600 lux
Fruitbody development:
Temperature:13-18 ℃
Relative humidity: 90-95%
CO_2: 2000-4000 ppm
Light requirement: 400-600 lux

Common name: Wood Blewit

Scientific name: *Clitocybe nuda* (Bull.:Fr.) Bigelow & Smith

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:10-15 ℃

Relative humidity: 95-100%

CO_2: ＜1000 ppm

Light requirement: 500-1000 lux

Fruitbody development:

Temperature:10-15 ℃

Relative humidity: 90-95%

CO_2: ＜2000 ppm

Light requirement: 500-1000 lux

Common name: Sunny mushroom, Brazil mushroom, Royal sun Agaricus, ABM, Almond Portobello

Scientific name: *Agaricus blazei* Murrill

Growth parameters:

Incubation temperature: 21-27 ℃

Primordia formation:

Temperature:21-24 ℃

Relative humidity: 80-90%

CO_2: 400-800 ppm

Light requirement: 100-200 lux

Fruitbody development:

Temperature:24-27 ℃

Relative humidity: 75-85%

CO_2: ＜2000 ppm

Light requirement: 100-200 lux

Common name: Reishi, Ling Zhi
Scientific name: *Ganoderma lucidum* (Curtis:Fr.) P. Karst
Growth parameters:
Incubation temperature: 21-27 ℃
Primordia formation:
Temperature:21-27 ℃
Relative humidity: 95-100%
CO_2: 2000-5000 ppm
Light requirement: 500-1000 lux
Fruitbody development:
Temperature:21-27 ℃
Relative humidity: 90-95%
CO_2: ＜2000 ppm
Light requirement: 750-1500 lux

Common name: Maitake (Dancing mushroom), Hen-of-the-woods

Scientific name: *Grifola frondosa* (Dicks:Fr.) S. F. Gray

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:10-20 ℃

Relative humidity: 90-95%

CO_2: 2000-5000 ppm

Light requirement: 100-500 lux

Fruitbody development:

Temperature:13-18 ℃

Relative humidity: 75-85%

CO_2: ＜1000 ppm

Light requirement: 500-1000 lux

Common name: Lion's mane mushroom, Monkey's head

Scientific name: *Hericium erinaceus* (Bulliard :Fries) Persoon

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:10-16 ℃

Relative humidity: 95-100%

CO_2: 500-700 ppm

Light requirement: 500-1000 lux

Fruitbody development:

Temperature:18-24 ℃

Relative humidity: 90-95%

CO_2: 500-1000 ppm

Light requirement: 500-1000 lux

Common name: Wood ear, Jew's ear

Scientific name: *Auricularia polytricha* (Montagne) Saccardo

Growth parameters:

Incubation temperature: 24-30℃

Primordia formation:

Temperature: 12-24℃

Relative humidity:90-100%

CO_2:600-1000 ppm

Light requirements: 500-1000 lux

Fruitbody development:

Temperature: 21-30℃

Relative humidity:85-90%

CO_2:1000-1500 ppm

Light requirements: 500-1000 lux